

PE SCREENING TEST FOR HUMAN BLOOD

A. SCOPE

A.1 Once a stain has tested positive for the presumptive presence of blood, determining if it is of human origin may follow. The *One Step* ABACard® HemaTrace® test is an immunochromatographic one-step test for the detection of human blood. In this test procedure a specimen is added to the sample well, "S", and allowed to soak in. If human hemoglobin (hHb) is present in the specimen, it reacts with the mobile monoclonal antihuman Hb antibody and a mobile antigen-antibody complex is formed. This mobile antibody-antigen complex migrates through the absorbent device towards the test area "T." In the test area "T" an immobilized antihuman Hb antibody is present. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. The conjugated pink dye particles concentrate in a narrow zone on the membrane. When the hHb concentration exceeds 0.05µg/mL the pink dye particles will form a pink colored band in the test area "T" indicating a positive result. In the area marked "C" there is stationary antiimmunoglobulin antibodies which bind excess monoclonal antihuman Hb antibodies that do not bind to the antibodies in area "T". The captured pink dye particles will form a pink colored band in the control area "C" indicating the test worked properly.

B. QUALITY CONTROL

- B.1 Each new lot number must be tested with a known positive and negative control before use.
- B.2 Results must be documented in the Laboratory Asset Management System (LAM).
- B.3 A positive control must be tested each day of use prior to or in conjunction with the testing of any unknown or suspected blood samples.
- B.4 Results of the day of use quality control testing must be documented in the case notes and include the lot#, expiration date and the positive control utilized.
- B.5 If the used quality control measures do not produce the expected result, the test will not be used on evidentiary samples and troubleshooting will be performed. New solutions or materials may be required.

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C. SAFETY

- C.1 Treat all biological samples as potentially infectious. Gloves, a face mask, eye protection (e.g. safety glasses or a face shield) and a lab coat must be worn.
- C.2 Appropriate manufacturer's product insert must be read prior to performing this procedure for the first time.
- C.3 Distinguish all waste as general, biohazard or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

- D.1 A known human bloodstain is used to test the cards.
- D.2 *One Step* ABACard® HemaTrace® test cards
- D.2.1 These cards may be used until depleted; however, the cards must be discarded on the expiration date. Storage will be according to manufacturer's recommendations.
- D.3 The buffer provided with the *One Step* ABACard® HemaTrace® test cards must be stored according to the manufacturer's recommendations.

E. EQUIPMENT

- E.1 Microcentrifuge tubes
- E.2 Pipettes or dropper
- E.3 Vortex
- E.4 Timer

F. PROCEDURES

- F.1 Remove the test device and the dropper from the sealed pouch.
- F.2 Label the device.

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- F.3 Place a small cutting (~1-2 mm²) of the stain into a microcentrifuge tube containing approximately 250 uL of the provided buffer and allow soaking until a noticeable light straw color change occurs. Vortexing may be performed to assist with stain extraction. The volume of buffer may be increased or decreased according to the intensity of the stain. (e.g. increased buffer volume for a heavy red-brown stain)
- F.4 Add 150 µL (4 drops using the manufacturer's supplied dropper) of the buffer containing the extracted stain into the sample well "S."
- F.5 Read result in 10 minutes. Positive results can be seen as early as 2 minutes depending upon the hHb concentration. Negative results must be given the entire 10 minutes.
- F.6 The high dose hook effect may occur when the human hemoglobin concentration is too high. An excessive amount of human hemoglobin not only binds to the mobile monoclonal antibody to form an antigen-antibody complex, but also migrates towards the test area "T." This free hHb blocks the antibody in the test area and therefore the mobile antigen-antibody complex with the pink color cannot bind to the antibody. As a result no pink line will form in the test area. Therefore, all stains which are phenolphthalein positive, Hematrace® negative and soaked producing a moderate to dark in colour buffer should be reexamined after diluting 1:10 to 1:100 with water or buffer.

G. INTERPRETATION GUIDELINES

- G.1 If there are two pink lines, one each in the test area "T" and in the control area "C," the test result is positive and indicates that the human hemoglobin level is at or above 0.05 µg/mL.
- G.2 If there is only one pink line (in the control area "C"), the test result is negative. This may indicate that no human blood is present, the human hemoglobin level is below 0.05 µg/mL, or the high dose effect is present (false negative).
- G.3 If there is no pink line visible in the control area "C", the test is inconclusive. Repeat the test and reexamine the test procedure carefully.
- G.4 Positive results may be obtained with primate (anthropoidea) and ferret blood.

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H. REFERENCES

- H.1 *A Compendium of Forensic Science Methods*, The Forensic Sciences Foundation, Inc., Colorado, 1980, page 31-40
- H.2 Gaensslen, R.E. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, US Government Printing Office, Washington, D.C., 1983, page 131-133, 215-244
- H.3 Spaulding, R.P., and Cronin, W.F., *Technical and Legal Aspects of Forensic Serology: A Laboratory Manual*, FBI Laboratory
- H.4 *One Step ABACard® HemaTrace® Test for the Forensic Identification of Human Blood* Technical Information Sheet, 2013

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